A Complete Enzymatic Recovery of Ferulic Acid From Corn Residues With Extracellular Enzymes From *Neosartorya spinosa* NRRL185

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Received 22 March 2006; accepted 2 June 2006

Published online 17 August 2006 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/bit.21056

Abstract: An economic ferulic acid recovery from biomass via biological methods is of interest for a number of reasons. Ferulic acid is a precursor to vanillin synthesis. It is also a known antioxidant with potential food and medical applications. Despite its universal presence in all plant cell wall material, the complex structure of the plant cell wall makes ferulic acid recovery from biomass a challenging bioprocess. Previously, without pretreatment, very low (3–13%) recovery of ferulic acid from corn residues was achieved. We report here the discovery of a filamentous fungus *Neosartorya spinosa* NRRL185 capable of producing a full complement of enzymes to release ferulic acid and the development of an enzymatic process for a complete recovery of ferulic acid from corn bran and corn fibers. A partial characterization of the extracellular proteome of the microbe revealed the presence of at least seven cellulases and hemicellulases activities, including multiple iso-forms of xylanase and ferulic acid esterase. The recovered ferulic acid was bio-converted to vanillin, demonstrating its potential application in natural vanillin synthesis. The enzymatic ferulic acid recovery accompanied a significant release of reducing sugars (76–100%), suggesting much broader applications of the enzymes and enzyme mixtures from this organism.

Keywords: ferulic acid; ferulic acid esterase; vanillin; biomass; corn bran; corn fiber

INTRODUCTION

Biomass is a rich source for ferulic acid, which is universally present in all plant cell wall materials as a crosslinking agent, linking different polysaccharides chains and polysaccharides with lignin (Graf, 1992; Mathew and Abraham, 2004). Recovery of ferulic acid from biomass is of interest for a number of reasons. A high-valued flavor compound, vanillin, can be synthesized from ferulic acid through a microbial conversion (Priefert et al., 2001; Rosazza et al., 1995). Vanillin through microbial conversion can be labeled as natural under the current EU and US regulations, when ferulic acid is derived from a natural source (biomass) and the recovery method is mild (such as biological method). Ferulic acid is also an antioxidant, potentially useful as food additives and medicines (Mathew and Abraham, 2004; Ou and Kwok, 2004). This has stimulated great interests in developing enzymatic methods to recover ferulic acid from biomass (Bonnin et al., 2002; de Vries et al., 2000; Faulds et al., 2004, 2005; Saulnier et al., 2001; Yu et al., 2002). Consequent to these efforts, many novel ferulic acid esterases (FAEs) were discovered (Crepin et al., 2003, 2004; Garcia-Conesa et al., 2004; Rumbold et al., 2003; Shin and Chen, 2006; Topakas et al., 2003a,b, 2004, 2005; Wang et al., 2004). Despite its importance, FAE alone is not sufficient to release ferulic acid from the biomass matrix. Synergistic use of FAE and other hemicellulases, especially xylanase and arabinofuranosidase, is necessary. Recent activities in enzymatic recovery of ferulic acid from various biomass resources are summarized in Table I. The recovery process varied greatly with the nature of the biomass. A nearly complete recovery from wheat bran was obtained (Table I). In contrast, corn residues seemed to be particularly recalcitrant, without pretreatment, very low amount of ferulic acid (3–13%) was recovered.

In our previous study, using a *Fusarium proliferatum* FAE and *Thermomyces lanuginosus* xylanase, only about 35% of ferulic acid was recovered (Shin and Chen, 2006). Using extracllular enzymes from *Aspergilus niger* NRRL 3, a known FAE producer, the recovery of ferulic acid was similarly low. Only after pretreatment, such as autoclaving and flash explosion, was high percentage of ferulic acid recovered (90–95%, Table I). Corn residues are the most promising source of ferulic acid with the highest ferulic acid

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Contract grant sponsors: USDA; CSREES
Contract grant number: 2004-35503-1520

Table I. Release of ferulic acid from plant cell wall materials by hemicellulases.

Substrate	Ferulic acid (%.w/w)	Substrate concentration and enzymes	Release of ferulic acid (%)	Polysaccharide hydrolysis (%)	Reference
Wheat bran	—	1%(w/v), AFAE III & xylanase of *T. viride*	95	—	Faulds and Williamson (1995)
Wheat bran	—	1%(w/v), PFAEB & xylanase of *T. viride*	98	—	Kroon et al. (2000)
Wheat-WIP	0.49	0.5%(w/v), 4 hemicellulases, 8 hemicellulases	96.9, 97.5	16.9, 98.7(xylose)	de Vries et al. (2000)
Wheat bran	—	5%(w/v), FAE-II & xylanase from *Sporotrichum thermophile* (1.5 U FAE & 300 U xylanase)[a]	23	—	Topakas et al. (2003b)
Wheat bran	—	5%(w/v), FAE-I & xylanase from *S. thermophile* (4 U FAE & 100 U xylanase)[a]	92	—	Topakas et al. (2003a)
Wheat bran	—	2.5–3.0%(w/v), TsFaeC & xylanase of *Trichoderma viride*	66	—	Garcia-Conesa et al. (2004)
Wheat bran	0.288	1%(w/v), Ultraflo L (1U FAE & 2.43 xylanase)[a]	90	30 (sol. sugar)	Faulds et al. (2004)
Wheat bran	—	2%(w/v), NcFaeD & xylanase of *T. viride* (0.1 U FAE & 200 U xylanase)[a]	36	—	Crepin et al. (2004)
Wheat-WAIR	1.03	2%(w/v), FaeA/Xln11	29.2	—	Faulds et al. (2005)
Brewer's grain	0.187	2%(w/v), Enzymes of *Streptomyces avermitilis* CEC T3339 (4U FAE)[a]	43	—	Bartolome et al. (2003)
Brewer's grain	0.255	1%(w/v), Ultraflo L (1U FAE & 2.43U xylanase)[a]	83	—	Faulds et al. (2004)
Sugar beet pulp	0.8	1%(w/v), SP584 (0.5U FAE & 37.5U xylanase)[a] *A. niger* I-1472 enzymes (5U FAE & 75U xylanase)	49.4, 38.7	28.6 (xylose), 22.0 (xylose)	Bonnin et al. (2002)
Oat hulls (≤250 μm)	0.38	2%(w/v), *Aspergillus* FAE & *Trichoderma* xylanase (Finnfeeds Inernational) (26U FAE & 32,768 U)	68.5	—	Yu et al. (2002)
Corn bran-SFE	3.1	2%(w/v), Novozyme 342 (9U FAE & 419 U xylanase)[a]	49.3, 95.3	80 (sol. sugar)	Saulnier et al. (2001)
Corn bran	3.1	1%(w/v), Novozyme 342 (0.5U FAE & 62.6U xylanase)[a]	13.6, 3.4	—, —	Bonnin et al. (2002)
Corn bran-autoclaved	3.4	1%(w/v), Novozyme 342 (0.5U FAE & 62.6U xylanase)[a]	31.9, 90.3	23 (xylose), 29.5 (xylose)	Bonnin et al. (2002)
Corn fiber	1.85	2%(w/v), *Neosartorya spinosa* NRRL 185 (10U FAE & 23U xylanase)[a]	99.5	97.3 (reducing sugar)	This study

[a]The enzyme load was calculated based on the enzyme units per gram of substrate.

WIP, water-insoluble pentosan from wheat; WAIR, wheat bran-alcohol-insoluble residues; FaeA, feruloyl esterase A from *A. niger*; Xln11, family 11 xylanase from *T. viride*; SP-584, cell wall-degrading enzymes from *Aspergillus* sp. (Novozymes, Bagsvaerd, Denmark), Novozyme 342 cell wall-degrading enzymes from *Humicolar insolens* (Novozymes, Bagsvaerd, Denmark); Ultraflo L, multifunctional β-glucanase from *H. insolens* (Novozymes, Bagsvaerd, Denmark); TsFaeC, feruloyl esterase C from *Talaromyces stipitatus* CBS 375.48; AFAE III, ferulic acid esterase (FAE-III) from *Aspergillus niger*; PFAEB, feruloyl esterase B from *Penicillium funiculosum*; FAE-II, feruloyl-esterase-II from *Fusarium oxysporum*; FAE-I, feruloylesterase-I from *Fusarium oxysporum*; NcFaeD, feruloyl esterase D from *Neurospora crassa*; Corn bran-SFE, corn bran soluble fraction after flash explosion.

content (3% by weight). However, the low recovery yield or the requirement for pretreatment greatly limits their many potential applications.

In our search for a more efficient method for ferulic acid recovery, a fungal strain, *Neosartorya spinosa* NRRL185, was discovered to have the capability to produce a full complement of enzymes necessary to liberate completely ferulic acid from corn bran and corn kernel skins, referred as corn fibers. We report here the successful use of its extracellular enzymes for the complete recovery of ferulic acids from corn bran and corn fibers and a partial characterization of the extracellular proteome.

MATERIALS AND METHODS

Corn Fiber Preparations

Forty-five kilograms of Corn of No. 2 yellow dent corn hand was soaked overnight in cold water and ground in a disc mill (Young Machinery Co., Muncy, PA). Hot water was added to ground corn in a stirred tank. Mash was heated with a steam sparger to 80°C while adding 200 mL of Spezyme Delta AA (Genencor, Rochester, NY) diluted with 800 mL of distilled water at 3 mL/min. After 1 h at 80°C, mash was cooled to 55°C, pH was adjusted from 6 to 4.4 with 20% sulfuric acid, and 200 mL Optidex L-300 (Genencor) was added. Final mash volume was 200 L. After 2 h at 55°C, insoluble solids were collected on a Hydrasieve (The Bauer Bros. Co., Springfield, OH) having an inclined screen with six wires per inch. Solids were washed by mixing with hot water (200 L) and washed insoluble solids were collected on the Hydrasieve. Weight of air-dried solids was 7.7 kg (17%). Starch and protein contents of solids were 39% and 14%. Corn fiber (lighter fraction) was obtained after separation in an aspirator, Model 6DT4-1 (Kice Industries, Inc., Wichita, KS).

Microorganisms

Neosartorya spinosa NRRL 185, isolated from teakwood (tropical hardwood) were used in this study.

Cell Growth and Preparation of Concentrated Crude Enzyme

The strain glycerol stock (15%, v/v) were maintained at −80°C after 1 day cultivation at 30°C with a YPD medium, which contained 1%(w/v) yeast extract, 2%(w/v) peptone, and 2.0%(w/v) glucose. The medium for enzyme production (Asther et al., 2002) contained (per liter): 0.2 g K_2HPO_4, 0.013 g $CaCl_2$, 0.5 g $MgSO_47H_2O$, 0.5 g yeast extract, 1.842 g diammonium tartrate, and 20 g corn bran (Canadian Harvest Process Ltd., ON, Canada). The fungal strain was cultivated in 500-mL Erlenmeyer flasks (100 mL of medium) in a shaking incubator (250 rpm) at 30°C. After 5 days, the cells were removed from the culture broth by centrifugation (10,000 rpm, 20 min), and the resulting supernatant solution was collected as a crude enzyme preparation. The proteins in the supernatant were concentrated 10–50 times by an ultrafiltration system using a Pellicon 10,000 MWCO membrane (Millipore, Bedford, MA).

Enzyme Assays

FAE activity was assayed by analyzing the free ferulic acid released from methyl ferulate (Apin Chemicals, Oxon, UK). The assay was routinely carried out at 40°C for 30 min in a 50 mM Na-phosphate buffer (pH 7.0) containing 1 mM methyl ferulate, and the liberated free ferulic acid was analyzed by HPLC. One unit of FAE activity was defined as the amount of enzyme that liberates 1 μmol ferulic acid per min.

Xylanase and endo-β-1,4-glucanase (CMCase) activities were determined in a 50 mM Na-phosphate buffer (pH 6.0), containing 1.0 % (w/v) boiled oat spelt xylan and sodium carboxymethylcellulose (Sigma, St. Louis, MO), respectively. After 30-min incubation at 50°C, the reducing sugar liberated in the reaction mixture was measured by the dinitrosalicyclic acid method (Chaplin, 1986). One unit of xylanase or CMCase activity is defined as the amount of enzyme that produces 1 μmol of reducing sugar as xylose or glucose per min.

α-Arabinofuranosidase, acetyl xylan esterase, and β-xylosidase activities are measured using *p*-nitrophenyl(PNP)-α-arabinofuranoside, *p*-nitrophenyl-acetate, and *p*-nitrophenyl-β-xylose (Sigma Chemical Co.) as the substrate, respectively. Hydrolysis of each PNP-substrate is determined by the release of *p*-nitrophenol. The reaction is carried out in a mixture containing 20 μL 20 mM PNP-substrate, 880 μL 50 mM sodium acetate buffer (pH 6.0), and 100 μL enzyme solution at 40°C. The reaction is stopped by adding 1 mL 2% (w/v) Na_2CO_3, and the optical density at 410 nm is measured. One enzyme unit is defined as the amount of enzyme releasing 1 μmol of *p*-nitrophenol per minute.

Determination of Ferulic Acid Content in Corn Bran and Corn Fiber

Corn bran or corn fiber (100 mg) was suspended in 2 M NaOH (5 mL) and incubated at 45°C. Samples (0.5 mL) were taken at different time intervals, acidified with HCl to pH 2, and extracted with ethyl acetate (0.3 mL) for five times. The ethyl acetate layers were combined, washed with water, and evaporated to dryness. Residues were dissolved in 0.5 mL methanol/water (50:50, v/v) and analyzed for ferulic acid by HPLC.

Enzymatic Recovery of Ferulic Acid

Corn bran or ground powders of corn fiber (≤425 μm) were suspended in a phosphate buffer (pH 7.0), a crude enzyme preparation of an appropriate volume was added, and the mixture was incubated at 45°C. Samples were taken at different time intervals, inactivated by adding acetic acid.

DOI 10.1002/bit

The supernatant was collected by centrifugation (13,000*g*, 10 min) and analyzed for ferulic acid by HPLC. To prepare ferulic acid for production of vanillin, ferulic acid was extracted with an equal volume of ethyl acetate for five times. The organic solvent layers were combined, washed with water, and evaporated to dryness in a chemical hood. The residue was dissolved in dimethylformamide.

Conversion of Ferulic Acid Into Vanillin by *Streptomyces setonii* ATCC391161

For bioconversion of ferulic acid into vanillin, *Streptomyces setonii* ATCC391161 (Sutherland et al., 1983) was cultivated at 37°C for 24 h in a shaker using the growth medium, which contained per liter: 4.65 g K_2HPO_4, 1 g KH_2PO_4, 0.2 g NaCl, 1 g yeast extract, 0.2 g $MgSO_47H_2O$, 0.05 g $CaCl_2$, and 4 g glucose. Bioconversion was initiated by adding ferulic acid to the cell culture to a final concentration of 1 μmol/mL. Temperature 37°C and a shaking speed 250 rpm were used. After 12 h, samples were taken for measurement of both ferulic acid and vanillin by HPLC.

Analytical Methods

Protein concentration was determined by the Bradford method (Bradford, 1976) using a commercial kit (Bio-Rad, Hercules, CA), in which bovine serum albumin was used as the standard. 10–20% gradient PAGE Ready gel and 7.5% native PAGE Ready gel (Bio-Rad) were used in SDS–PAGE and native-PAGE analysis.

The phenolic acid analysis was performed by HPLC on a LiChrospher 100 RP-18 column (Agilent Technologies, Palo Alto, CA). Detection was carried out at 280 nm based on calibration curves prepared using standard ferulic acid or vanillin (Sigma). A gradient elution was used with 0.01% acetic acid (A) and methanol (B) at a flow rate of 1.0 mL/min. A linear gradient was run as follows: $t = 0$ min, 80:20 (A:B); $t = 4$ min, 80:20; $t = 24$ min, 60:40; $t = 27$ min, 0:100; $t = 29$ min, 0:100; $t = 30$ min, 80:20; $t = 35$ min, 80:20.

The glucosamine content in the dried biomass was used as a measure of cell mass. The dried biomass was hydrolyzed with 6 N HCl for 20 min at 120°C. The amount of glucosamine in the acid hydrolysate was then determined using the Morgan-Elson method (Chaplin, 1986).

RESULTS

Extracellular Cellulases and Hemicellulases Production by *Neosartorya spinosa* NRRL185

Neosartorya spinosa NRRL185 was discovered in our screening process as a microbe capable of producing extracellular enzymes to liberate ferulic acid from corn bran and corn fibers. The extracellular proteome was then studied by following the various enzyme activities while cells were grown on corn bran. Six enzyme activities were detected, including cellulases as determined using sodium carboxymethylcellulose as substrate, hemicellulases (xylanases, β-xyloxidase, FAE, α-arabinofuranocidase, and xylan acetylesterase). Another enzyme, α-glucuronicidase was also detected at a very low level $\leq$1.5 units/L). A typical time profile of cellulase and hemicellulase production is shown in Figure 1. The production of xylanase and α-arabinofuranosidase by *Neosartorya spinosa* NRRL 185 was found to be strongly growth dependent and peaked at Day 3 (43 units/L and 41 units/L) just before the biomass reached its highest level at Day 4. Their activities were subsequently decreased until the end of the cultivation. FAE activity was first detected at Day 4, and its production continued until leveled off at Day 8 (34 units/L). The synthesis of β-xylosidase started at the onset of the cultivation and continued throughout the cultivation. As a result, β-xylosidase became one of the dominant enzyme activities at the end of the cultivation (Day 8 and Day 9). In addition, low activities of cellulases and acetyl xylan esterases were detected (less than 10 units/L).

Characteristics of Crude Hemicellulase at Different Cultivation Time

Because of each enzyme's distinct dynamics of production, the extracellular enzyme compositions varied significantly with cultivation time, with an earlier domination by xylanase and α-arabinofuranosidase shifted to a later domination by β-xylosidase and FAE (Fig. 1 and Table II). Further characterizations were carried out on enzyme preparations from Days 5, 7, and 9. Figure 2 shows the SDS–PAGE and native-PAGE of these three samples. Enzyme patterns for samples collected at Days 5 and 7 were similar except that the Day 5 sample showed an additional band corresponding to a protein about 50 kDa. This additional band also appeared in

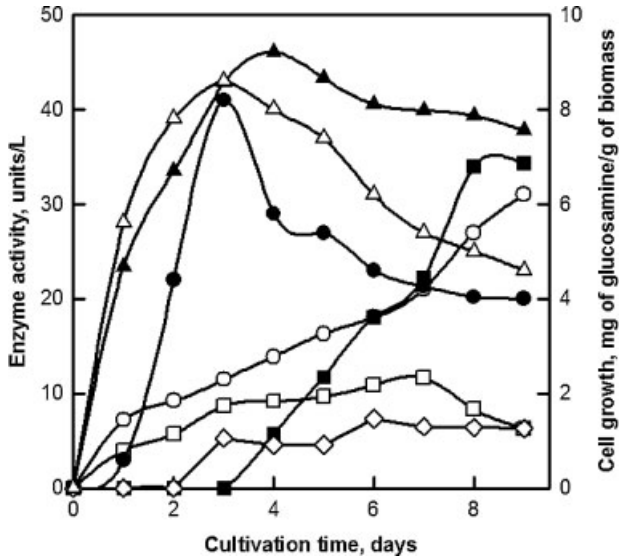

Figure 1. Hemicellulase production and cell growth of *Neosartorya spinosa* NRRL185. *Neosartorya spinosa* NRRL185 was cultivated in a medium containing 2% (w/v) corn bran at 30°C and 250 rpm. Symbols: ▲: cell growth; ■: feruloyl esterase; △: xylanase; ○: β-xylosidase; ●: α-L-arabinofuranosidase; □: acetyl xylan esterase; ◇: CMCase.

Table II. Hemicellulase composition of crude enzyme harvested at different cultivation time.

	Specific activity (units/mg protein)		
Enzymes	5 Days	7 Days	9 Days
Xylanase	0.33 ± 0.07	0.30 ± 0.05	0.13 ± 0.05
CMCase	0.05 ± 0.02	0.10 ± 0.05	0.02 ± 0.02
Feruloyl esterase	0.14 ± 0.02	0.32 ± 0.03	0.21 ± 0.04
α-Arabinofuranosidase	0.24 ± 0.05	0.10 ± 0.04	0.04 ± 0.02
Acetylxylan esterase	0.11 ± 0.02	0.14 ± 0.03	0.08 ± 0.03
β-Xylosidase	0.17 ± 0.03	0.22 ± 0.05	0.32 ± 0.04

the native gel. The sample from Day 9, however, was quite different from the two previous samples. Less distinct bands could be discerned.

In order to identify xylanase, α-arabinofuranosidase, and FAE on the native-PAGE for the Day 5 sample, three identical gels were prepared and each native-PAGE gel was divided into seven groups, A through G, as shown in Figure 3. The excised gel fragments were cut into small pieces, and then incubated separately with an appropriate substrate at 40°C for 4 h. The product from each respective reaction was then identified and used as an indication for the presence of a particular enzyme activity. Specifically, the released reducing sugar from xylan was detected with dinitrosalicyclic acid method, which showed a brownish color in the presence of xylanase activity. The ferulic acid liberated from methyl ferulate was identified by HPLC. For α-Arabinofuranosidase, the cleavage of *p*-nitrophenyl-α-arabinofuranoside generated a yellow color, indicative of the α-Arabinofuranosidase. As shown in Figure 3, xylanase and FAE activity was found in groups A, B, C, and D. However, α-arabinofuranosidase was found in Group C only. It appears that there was only one α-arabinofuranosidase present, whereas four xylanases and four FAE activities were present in the extracellular proteome. Further, xylanase and FAE were closely associated with each other. In one case, the Group C, three activities were found with the single gel piece. Due to limited resolution of the native gel, it is not clear whether a protein complex is formed or these represent multi-functional enzymes. Further proteomic studies are needed to understand the exact nature of the association.

Recovery of Ferulic Acid From Corn Bran and Corn Fibers

Figure 4 shows a time course of ferulic acid release from corn fiber by crude enzymes collected from the growth media after five days of cultivation. After 12 h reaction, more than 80% of the alkaline extractable ferulic acid was released, and nearly 100% recovery of ferulic acid was reached after 24 h. Despite higher FAE activities with the Day 7 and Day 9 samples (Table II, Fig. 1), the release of ferulic acid from corn residues were much lower (data not shown), suggesting that concerted action by hemicellulases are more important than the activity of a single enzyme. 100% recovery of ferulic acid from corn bran was also achieved but it took longer time (48 h vs. 24 h) and more enzymes (twice as much). This seemed to be reasonable as corn bran contains more ferulic acid than corn fiber (3.2% vs. 1.85%, Table III).

The release of ferulic acid was strongly correlated with the liberation of reducing sugars from corn fiber (Fig. 4). The liberated reducing sugars from corn fiber were 814 mg/g of substrate. The corn fiber contains approximately 80% polysaccharide. Therefore, for corn fiber, a complete hydrolysis of polysaccharides was reached. Four major monosaccharides, glucose, galactose, xylose, and arabinose were each recovered nearly 100% (Table III). However, for the corn bran, 606 mg reducing sugar per gram of corn bran was measured; representing about 75.7% hydrolysis of polysaccharides at the time when 100% ferulic acid was liberated. While galactose and arabinose were 100%

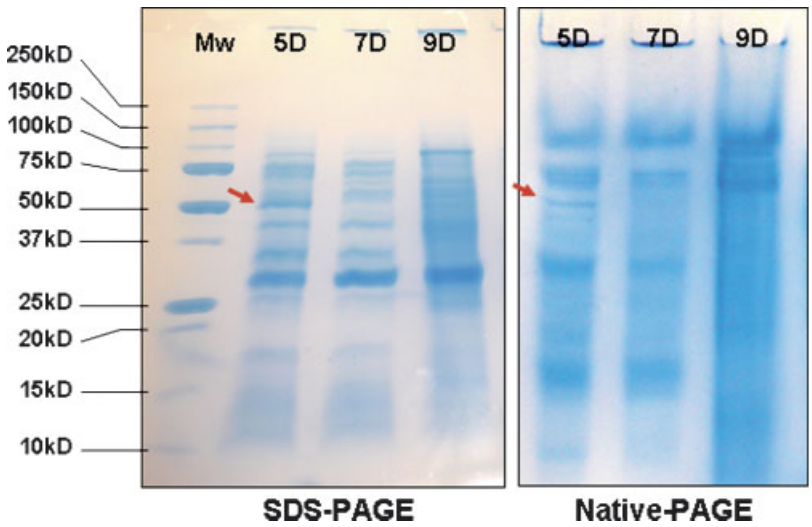

Figure 2. SDS-PAGE and native-PAGE analysis of extracellular proteins of *N. spinosa* NRRL185 at different harvest times. 20 μg protein of each sample was loaded onto the polyacrylamide gel for SDS-PAGE and native-PAGE, respectively. [Color figure can be seen in the online version of this article, available at www.interscience.wiley.com.]

DOI 10.1002/bit

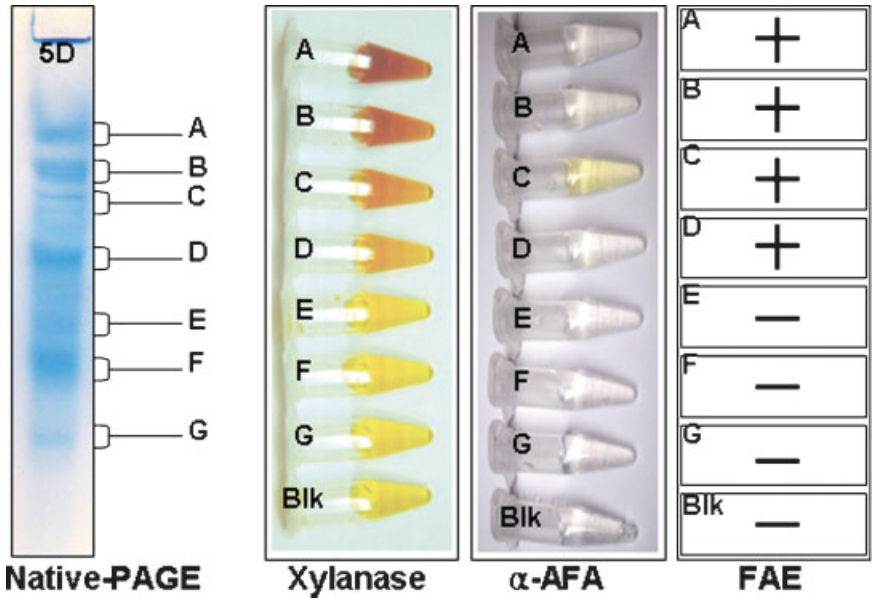

Figure 3. Identification of xylanase, α-arabinofuranosidase, and feruloyl esterase. Abbreviation: Blk, blank. [Color figure can be seen in the online version of this article, available at www.interscience.wiley.com.]

recovered, incomplete recovery was observed with xylose (87%) and glucose (44%). This difference may be due to the different make-up of polysaccharides in these two substrates.

Bioconversion of the Recovered Ferulic Acid to Vanillin by *Streptomyces setonii* ATCC391161

Ferulic acid recovered from corn fiber was extracted with organic solvent and was then tested as a potential starting material for vanillin production. *Streptomyces setonii* ATCC391161, a known microbe capable of converting ferulic acid to vanillin, was grown for 24 h, and ferulic acid was added into the of *S. setonii* culture to a final concentration of 1 mM to initiate the reaction. Figure 5 shows that ferulic acid was converted to vanillin, indicating that corn fiber is a suitable source of ferulic acid in the synthesis of vanillin. In a 12-h reaction, 98% ferulic acid was converted, 0.43 μmol vanillin was formed, corresponding to a yield of 43%. Substantial vanillic acid along with other un-identified by-products were formed. Further optimization with the bioconversion will lead to a better product yield.

DISCUSSION

Biomass represents a great challenge to bioengineers in the design of an enzymatic process. Due to the extremely complicated structure, often an array of enzymes with varied functions is necessary. In addition, conditions need to be provided to exploit the synergy among all enzymes. Further, because the number of enzymes involved is large, using purified enzymes to achieve the ratio, composition needed for

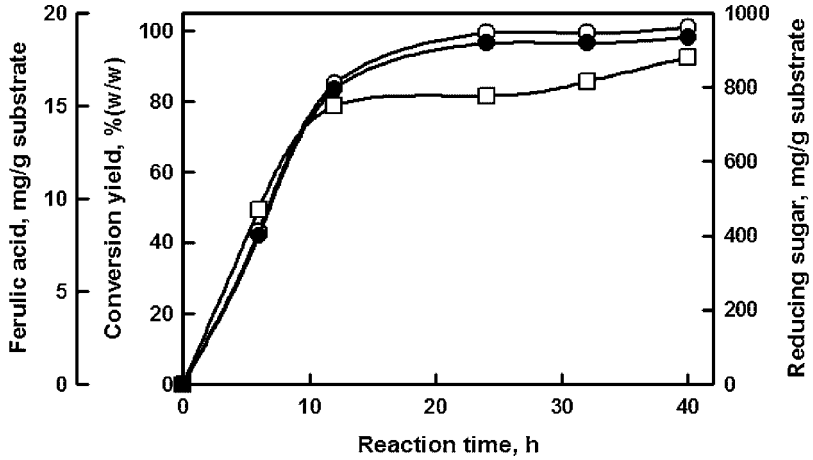

Figure 4. A typical time course of ferulic acid release from corn fiber by extracellular enzymes of *N. spinosa* NRRL185. Reaction conditions: 20 mg substrate/mL, 0.1 units FAE/ 10 mg substrate, 50 mM Na-phosphate buffer (pH 7.0), 40°C, 40 h and 250 rpm. Symbols: □: reducing sugar; ●: ferulic acid; ○: conversion yield.

Table III. Monosaccharide compositions of hydrolysates of corn bran and corn fiber.

Compound	Corn fiber (10 mg)		Corn bran (10 mg)	
	Chemical hydrolysis[a] (mg)	Enzymatic hydrolysis[b] (mg) (%)[c]	Chemical hydrolysis[a] (mg)	Enzymatic hydrolysis[b] (mg) (%)[c]
Glucose	4.76	4.73 (99.2)	3.18	1.41 (44.3)
Galactose	1.03	1.03 (100)	0.99	0.98 (99.0)
Xylose	1.15	1.14 (99.1)	2.68	2.33 (86.9)
Arabinose	1.24	1.24 (100)	1.35	1.34 (99.2)
Ferulic acid	0.185	0.184 (99.5)	0.322	0.318(98.8)

Data are averages of at least three independent experiments.

[a]Chemical hydrolysis of corn bran or corn fiber (10 mg) was carried out with 1 N HCl. Liberated monosaccharides were analyzed using Dionex BioLC system equipped with CarboPac PA-20 column.

[b]Enzymatic hydrolysis of corn bran or corn fiber (10 mg) was carried out at 40°C for 24 h (corn fiber) and 40 h (corn bran) with extracellular enzymes from *N. spinosa* NRRL185 (the amount of enzyme was adjusted based upon the FAE activity: 0.1 unit FAE for corn fiber and 0.2 unit FAE for corn bran).

[c]The percentage recovery yield of acid-extractable monosaccharides by enzymatic hydrolysis: $c = 100\% \times (b/a)$

a synergistic action is often practically impossible. Current understanding about enzyme compositions and relative ratios necessary for maximal synergy are not complete to guide a design and optimization of such an enzymatic process. The discoveries of microbes that produce a full complement of enzymes to liberate ferulic acid circumvent these difficulties. As illustrated in this study, by growing cells using the biomass of interest, a right mix of the extracellular enzymes with correct ratios for maximal synergy are produced, which could be used directly in biomass utilization after harvesting the extracellular enzymes. This approach represents a quicker and more economical method as it does not require elaborate post-harvest processing steps. An added advantage is that the process and the resultant products will be more likely to be embraced by consumers as the process involves wild type cells, not GMOs. This is particularly important for food products such as vanillin.

The ability of the enzymes to hydrolyze a significant amount of fermentable sugars (76–100%) from corn residues without prior treatment indicates potential, much broader applications of these enzymes in biomass utilization.

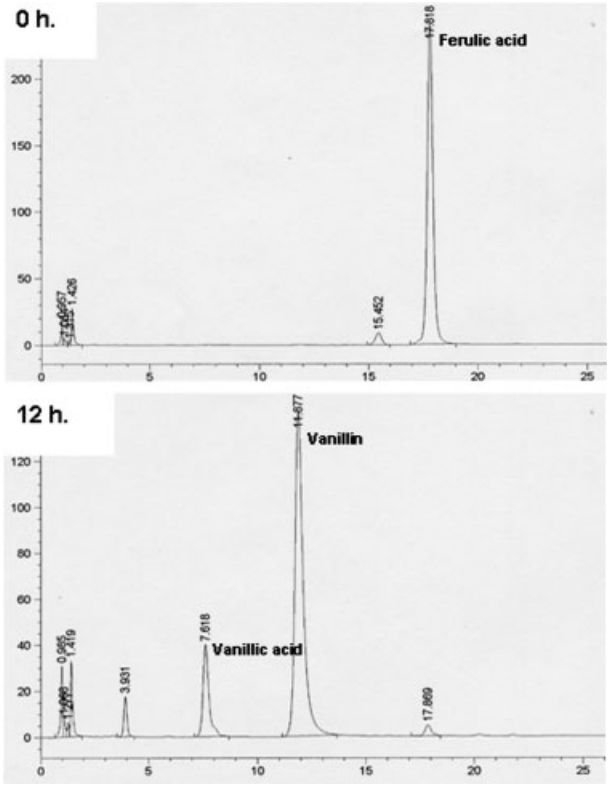

Figure 5. HPLC chromatograms of the reaction mixtures before and after the bioconversion of recovered ferulic acid to vanillin.

This research is funded through a grant from USDA, CSREES 2004-35503-1520. We thank Sunopta Ingredients for providing corn bran for this study.

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